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Award Number: DAMD17-99-1-9169

TITLE: The Involvement of Human Cyr61 in Heregulin Induction of Breast Tumor Progression

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REPORT DATE: August 2002

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2002	Annual Summary (1 Aug 01 - 31 Jul 02)

4. TITLE AND SUBTITLE
The Involvement of Human Cyr61 in Heregulin Induction of Breast Tumor Progression

5. FUNDING NUMBERS
DAMD17-99-1-9169

6. AUTHOR(S):
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

This fellowship initially concerned the role of the cytokine, heregulin, in the regulation of hormone receptor status in breast cancer. The mechanism by which breast cancer progresses from an ER+ to an ER- is of considerable clinical importance because some estrogen receptor-positive (ER+) breast tumors may progress to ER-negative and/or to deadly metastatic diseases. The justification for changing the project so late in the term is that Dr. Ruth Lupu, the mentor on the original award, left the LBNL shortly after I moved to the US to join the lab. Personal circumstances prevailed in my decision to change my mentor to Dr. Mary Helen Barcellos-Hoff and stay at LBNL. Recent studies in Dr. Barcellos-Hoff lab showed that depletion of transforming growth factor-beta (TGF-β), in the *Tgfβ1* null mouse mammary gland leads to increased frequency of proliferating ER+ cells, and indeed increased numbers of ER+ cells as detected by immunofluorescence. Therefore, we proposed to change the focus of the award from heregulin to TGF-β, which is also known to regulate heregulin. In the last 3 months, I have shown by Western blot analysis of mammary gland extracts of wild type and *Tgfβ1* heterozygotes mice that the ER level is higher in heterozygotes than wild type, consistent with the immunofluoresence data. As a continuation of this work on ER regulation, I will investigate how TGF-β1 suppresses ER using primary mouse mammary cell culture.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, transforming growth factor-β, estrogen receptor, ERα	26
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

This report covers the period December 2001 to September 2002. This post-doctoral training grant was originally funded for the project titled "The Involvement of Human Cyr61 in Heregulin Induction of Breast Tumor Progression" with Dr. Miaw-Sheue Tsai as the PI under the mentorship of Dr. Ruth Lupu. The fellowship was transferred to me in December, 2001 under the mentorship of Dr. Ruth Lupu. Because my doctoral degree is in the area of pharmaceutical chemistry, I required an introduction to the key techniques in cell culture, biochemistry, and molecular biology that are necessary to conduct research in breast cancer. Accordingly, Dr Lupu assigned me to a phytomedicines research project that was ongoing in her laboratory to enable me to master the necessary techniques while dealing with fairly familiar research questions. By so doing I would smoothly transition into the biomedical research arena. The overall goal of that ongoing project was to evaluate natural products (herbal medicines/phytomedicines) either currently in the market or about to be introduced for alternative treatment of breast cancer and menopausal symptoms [specifically for women in whom estrogen replacement therapy (ERT) is contraindicated because they have other risk factors for breast cancer].

During this training period, the experiments conducted involved testing herbal extracts for their estrogenic effects and other biological properties. I was thus able to master techniques in cell culture, as well as assays e.g. Ishikawa assay useful for evaluating the estrogenicity of a potential breast cancer chemotherapeutic agent. I also learnt several cell proliferation assays that use non-radioactive techniques for assessing cell viability & proliferation (anchorage dependent growth) as well as soft agar assay, an *in vitro* technique useful for evaluating anchorage independent growth of cancer cells to assess their potential tumorigenicity. Other assays learnt included ERE-luciferase reporter assay, RNAse protection assay and DPPH assay, a robust bench top assay for assessing preliminary antioxidant activity of agents. Unfortunately, my mentor Dr. Lupu left LBNL in May, 2002 but I chose to stay. Nonetheless I have summarized this project as **Part I** of this report.

In June 2002 I requested that my mentor be changed to Dr. Mary Helen Barcellos-Hoff at LBNL and as a consequence we requested a change in research direction. As with the original proposal, the goal of this project is study the regulation of estrogen receptor (ER) regulation in breast cancer. However I will study TGF-β rather than heregulin, because preliminary data in

mouse mammary gland have revealed a new and exciting aspect of ER regulation: the frequency and proliferation of ER positive cells are regulated by TGF-β. Relevant to the original proposal, TGF-β also regulates heregulin. Thus, the global goal of the proposal, i.e. to understand how ER status is regulated, will be maintained but the focus will change from heregulin to TGF-β and the model will change from human cell culture to mouse mammary epithelial cells. The benefit of this project to myself as a scientist is that I have expanded my repertoire of techniques and understanding of breast cancer biology. The benefit to the DOD Breast Cancer Research Program is that ER populations in the human breast are known to increase with age and in tissue at increased risk of breast cancer. Therefore understanding the fundamental regulation of this population is imperative to understanding its dysregulation. We requested the change in the statement of work in July, 2002. **Part II** of this report summarizes the project titled "The Role of TGF-β in the Regulation of Estrogen Receptor During Mouse Mammary Development and Carcinogenesis" and work done during the 3 months (July –September 2002).

BODY

PART 1: Phytomedicines research project

Project: Black Cohosh (BC): A potential Herbal menopausal remedy

Back ground of the research: *Actaea racemosa* L., commonly known as black cohosh (BC) is a remedy currently being taken by many women as an alternative to estrogen replacement therapy (ERT) in order to alleviate menopausal symptoms, such as hot flashes. It is claimed to reduce the frequency of hot flashes. However, the mechanism by which it does so is still unknown. BC has been shown to possess estrogenic activity and recently anti-estrogenic activity was also reported (1, 2).

We tested these extracts for estrogenic activity using the Ishikawa cell assay that measures the estrogenic activity of compound(s) by inducing an endogenous alkaline phosphatase (AP) enzymatic activity in the Ishikawa cell line (3). MTS cell proliferation assay was used to determine the extracts' effects on the *in vitro* growth of MCF-7 and MDA-MB-231 breast cancer cells. The effects of these extracts on the anchorage-independent growth of breast cancer cells

were also assessed using the soft agar assay in which the ability of cells to form colonies would indicate tumorigenicity. At the molecular level, the ability of the BC extracts to modulate the estrogen receptor (ER) function was evaluated using the estrogen-responsive element (ERE)-luciferase reporter assay (4).

Results: BC extracts did not induce the transcriptional activation of the estrogen-responsive element (**Figure 1**), did not regulate the expression of estrogen-regulated genes in the RNase protection assay (**Figure 2**), had no effect on the growth of ER-positive breast cancer cells and lastly did not induce colony formation in ER-positive cells (**Figure 3**), These data corroborated well with the preliminary studies that had earlier been done on BC extracts in the lab. A manuscript partially based on these results was drafted and Dr Lupu is currently finalizing it for publication.

Taken together with preliminary data that had been collected earlier, this data demonstrated that no estrogen-like activity is present in any of the BC extracts tested. Therefore, BC roots and rhizomes appear safe for use as an herbal remedy for the treatment of hot flashes in menopausal women for whom estrogen replacement therapy would be contraindicated.

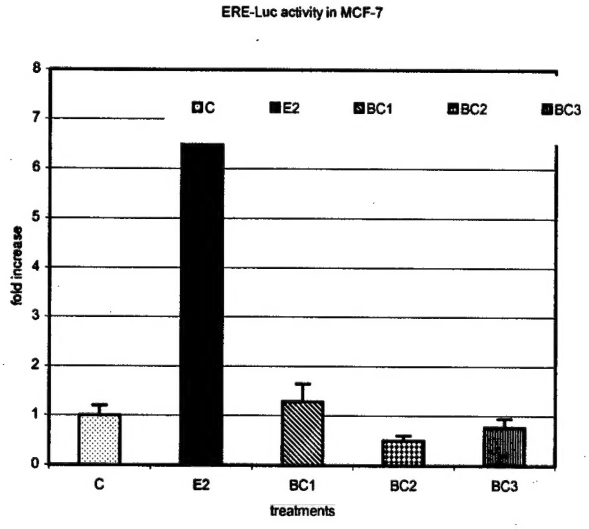


Figure 1. Effects of BC extracts on Estrogen-Responsive Element (ERE)-Luciferase activity: Cells cultured for four days in CCS were plated (100,000 per well) and allowed to attach overnight, then transfected with an estrogen-responsive luciferase reporter plasmid and an internal control plasmid PmCLV. Following transfection, cells were treated with E2 (1 nM) or BC extracts (20 μg/ml) for 24 hrs. The activity of the luciferases was measured using the Promega kit as per manufacturer's instructions. The results are expressed as fold increase over controls.

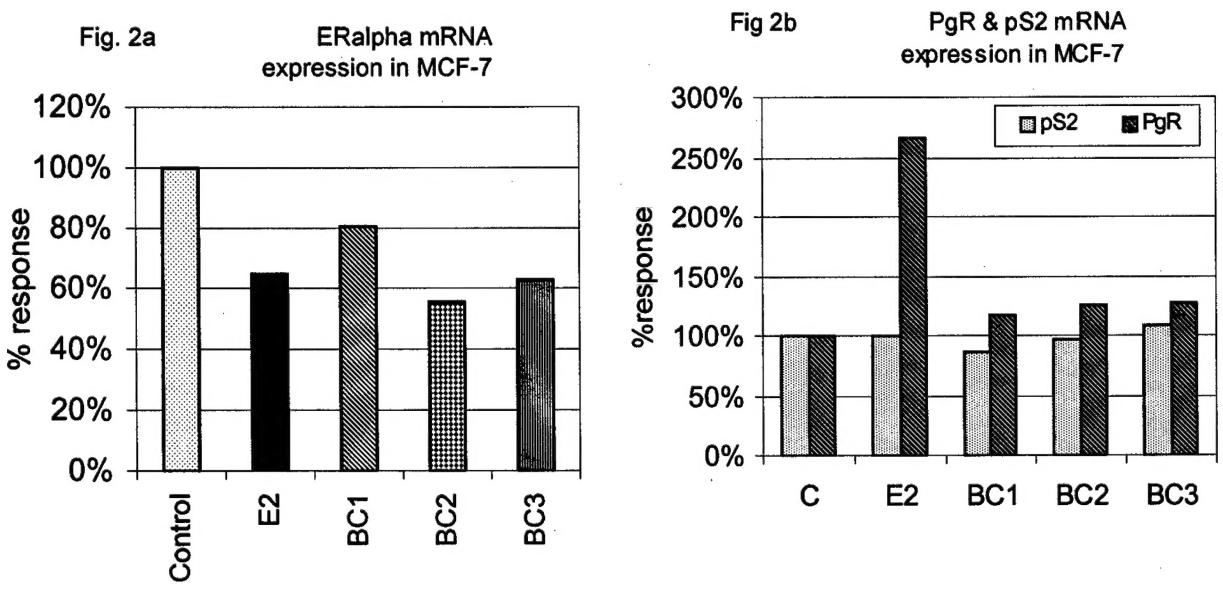


Figure 2 . a) Effects of BC extracts on ER alpha mRNA expression in MCF-7 Cells grown in CCS for 4 days were treated with the BC fractions for 24-h. Total cellular RNA was extracted using TriPure Isolation Reagent (12) and quantified by spectrometry. [^{32}P]-labeled ERα, pS2 or PgR riboprobes (50,000 counts/min) were hybridized with 30μg of sample, incubated at 55°C for 16h, and digested with RNAse (10.5, 4.67, 15 μg/sample for ERα, PgR and pS2, respectively) for 30 min at 28°C. The digestion was terminated by the addition of Proteinase K (1μg/ml) and 1% SDS. Samples were then extracted with phenol, precipitated along with 10 μg yeast tRNA in absolute ethanol, and where necessary also washed with 70% ethanol. The RNA was re-dissolved in denaturing loading buffer, electrophoresed on 6% polyacrylamide gel, and the protected fragments were visualized by autoradiography.

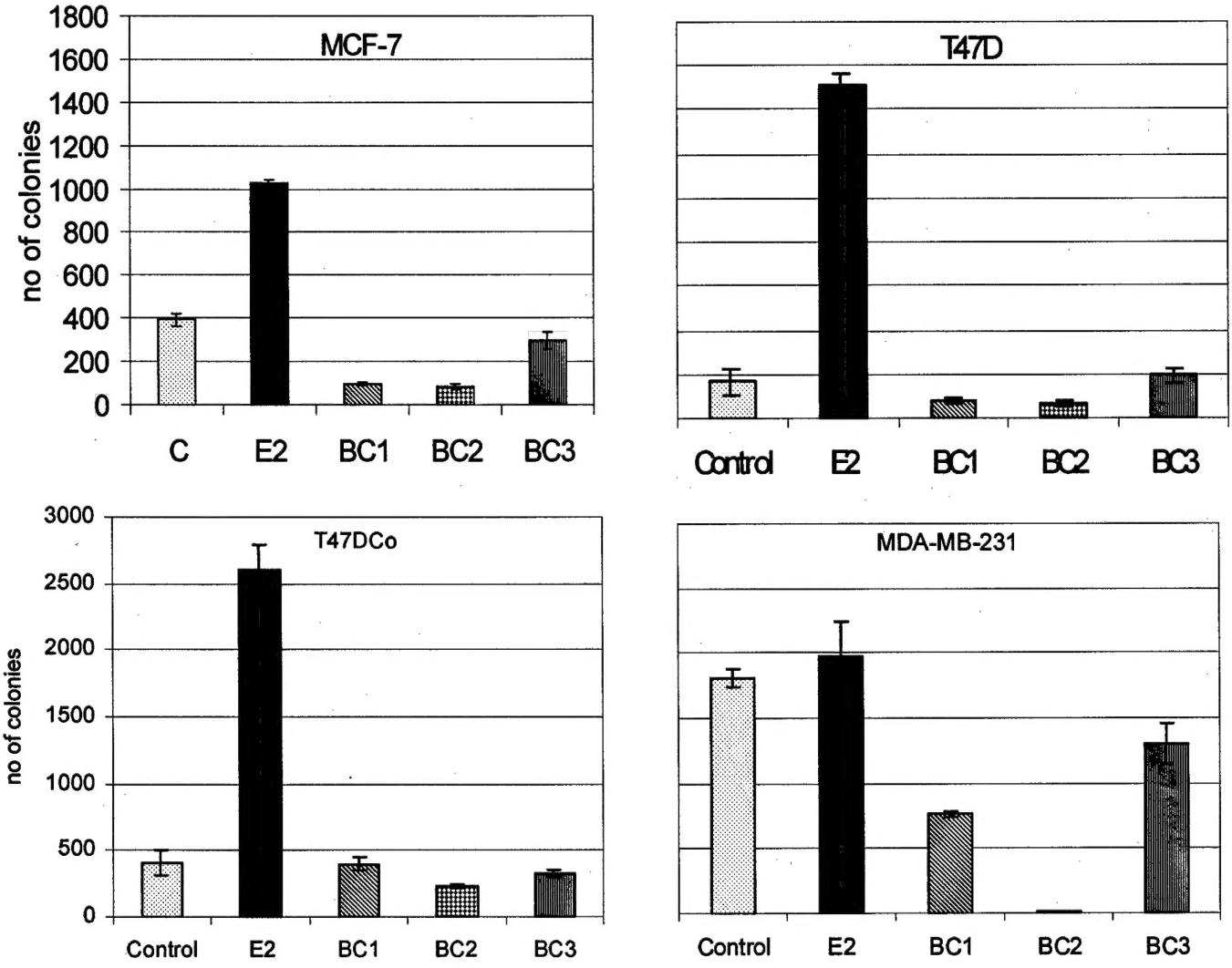


Figure 3 (a-d): Effects of BC extracts on the anchorage-independent growth of MCF-7 (a), T47D (b) T47Dco (c) and MDA-MB-231 (d) in soft agar assay. Four days before initiation of the experiment, cells were passed into phenol red-free IMEM supplemented with 5% CCS. The assay was performed as follows. 1.5mL of 0.6% agar suspended in IMEM media was layered as the bottom layer in a 6-well plate. Cells (20,000/ml in the case of MCF-7, T47D, T47DcO, T47D V22 and MDA-MB-453, and 10,000/ml in the case of MDA-MB-231 and MDA-MB-435) were suspended in 0.35% agar mixed with BC extracts at a concentration of 20μg/ml. After an additional 14-21 days of incubation (7days for MDA-MB-231 and MDA-MB-435) at 37°C, colonies were stained with 0.5ml of 1mg/ml Crystal Violet for 24 hour, and counted using the Accu-count 2000 colony counter .

BODY:

PART 1I "The Role of TFG-β in the Regulation of Estrogen Receptor During Mouse Mammary Development and Carcinogenesis"

Statement of Work

It is also well known that estradiol (E2) signaling through ER-α (one of the isoforms of ER) plays a central role in mammary epithelial cell proliferation. However a variety of recent studies have shown that estrogen receptor positive (ER+) cells do not proliferate. ER- cells usually proliferate and stain with proliferation markers such as Ki67 but are frequently located next to ER+ cells. Thus, although ER+ cells do not proliferate, they are necessary for proliferation, as shown by the lack of ductal outgrowth in the ER knockout mouse, and appear to regulate the proliferation of ER- cells via a paracrine mechanism.

Transforming growth factorβ1 (TGF-β) is the most potent inhibitor of human and mouse mammary epithelial cell proliferation known. Studies in our lab have shown that at estrus when there is a high rate of cell proliferation, nearly all ER+ cells co-localize with intense TGF-β staining, consistent with their non-proliferative status. It appear that TGF-β acts as a brake restraining the ER+ cells from proliferating while at the same time the ER+ cells, in response to hormonal stimulation by estrogen (E2), send out a signal to the ER- cells to proliferate. This hypothesis seems plausible given that when TGFβ1 level is reduced (as is the case in the *Tgfβ1* heterozygotes), ER+ cells proliferate more as evidence by the increase in the population of ER+ positive cells compared to the same population in the wild types. Understanding the role that TGFβ plays in the proliferation of ER+ cells is important because it is known that ER+ breast cancer can progress to more aggressive ER- negative cancer that is by its nature anti estrogen resistant and is more likely to become a deadly metastatic disease. The mechanism by which breast cancer progresses from the E2-dependent phenotype to the E2-independent one is not yet fully understood and yet it is important clinically as it would help identify possible targets of intervention in the control of or halting breast cancer progression.

Specifically understanding the role of TGβ in this process may unveil how TGFβ could be targeted in the control of breast cancer since it is well known that increased TGFβ activity is

associated with breast cancer progression (5) and can functionally mediate metastatic disease (6-8). The project will make use of *Tgfβ1* heterozygote Balb/c mice as a model and will include immunohistochemisrty studies, protein analysis and primary culture of mouse mammary tissue.

The specific objectives in this project are:

1) To substantiate the role of TGF-β in regulation of ER during mammary development I will determine the frequency of ER+ cells and the level of ER as a function of TGF-β activity in Balb/c mice *Tgfβ1*. null heterozygote and wildtype mice and compare these data to those previously obtained in the C57bl/129SV.

2) To determine whether TFGβ suppresses ER we will use primary mouse mammary epithelial and human breast cell cultures.

Preliminary work:

On embarking on this project the first task was to establish culture of MCF-7 cells in a serum free media. Our interest is to determine the level of ER in these cells and investigate how this level changes in the presence of various concentrations of TFG-β. Serum, a necessary component of regular growth media formulated to support the growth of these cells is also a rich source of TFG-β. As such it was necessary to develop a method of maintaining these cells in serum free medium in order to be able to investigate the effect of external TFG-βthat will be added in the media.

I have conducted Western blot protein analysis to determine the levels of ER in TFG-β +/+ (wild type) and TFG-β+/- (heterozygotes) mouse mammary glands at different stages of the estrus cycles. In addition I have done immunohistochemistry studies to determine the distribution of proliferating and ER+ cells in wild type and heterozygotes.

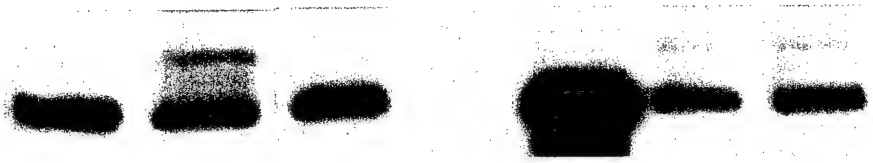
Results

Total protein lysates were prepared from mammary gland (MG) samples obtained from five wild-type TGF-β +/+ Balb/c mice. Included in the analysis, as controls, were uterine tissue lysates as positive control for ER and of the intestine tissue lysates as a negative control, both

were obtained from the same a wild-type Balb/c mice. The lysates were subjected to Western blot analysis and immunodetection for ER. The results (**Figure 4**) show that ER in the MG was about 10× less than that found in the uterus.

In a separate experiment we compared two extraction methods that were available in the laboratory, to determine which would give a suitable protein extract in which we would be able to detect ER using the less protein than reported in most research papers. The two protocols are arbitrarily denoted Sh and R in this discussion. The extractions were performed simultaneously in duplicate tissues and, the protein concentration in the lysates, determined by Bio-Rad DC protein assay method. Comparable protein concentrations were obtained with both protocols and total yield was also the same. There was a slight difference in the protein profiles in Sh and R protocols and a marked difference in the immunodetection sensitivity for ER when smaller amounts of proteins (20μg) were analyzed, with the R protocol proteins being more sensitive compared to Sh protocol proteins **(Figure 6).** We will therefore use the R protocol for all future protein extracts for ER detection.

ER alpha



Genotype	Wt	Wt	Wt	Wt	Wt	Wt	Wt
Protein qty (μg)	MG (20)	MG (20)	MG (20)	Int* (10)	Ut**(10)	MG (20)	MG (20)
Mice Strain	C57 Bl6	Balb/c	Balb/c	Balb/c	Balb/c	Balb/c	Balb/c
Lanes	1	2	3	4	5	6	7

Int*: Intestine

UT: Uterine**

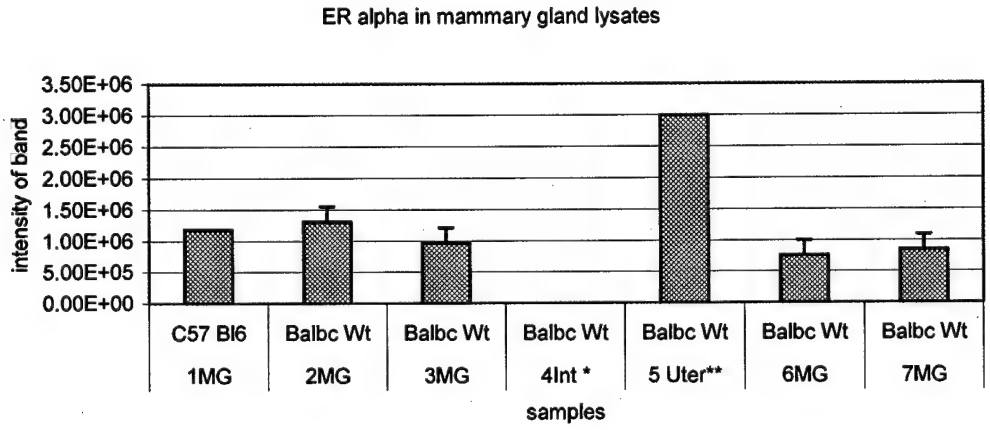
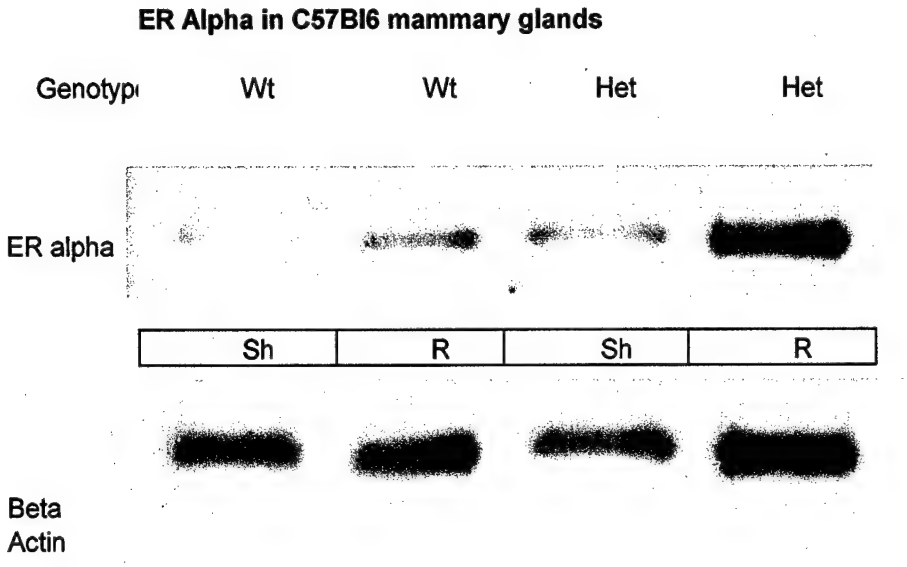
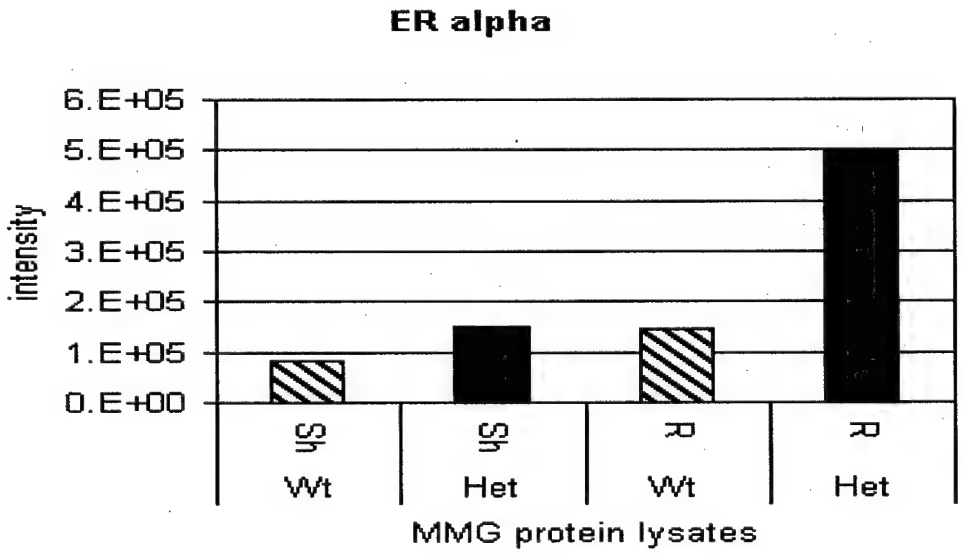


Figure 4 Densitometry of digitized films from Western blot analysis of Mammary Gland lysates. The mean intensity for the four Babl/c MG lysates was 1.5 $X10^6$units and the standard deviation was 16% of the mean value. In this experiment, our positive control shows a band at 67kD corresponding to the ER-α. The intestine lysate used as a negative control showed no band at this position.





Figures 5: Protein lysates were prepared using two different protocols denoted Sh and R. 20µg of these extracts were subjected to Western blot analysis. In Lane 1 & 3 are wild type and heterozygote lysates prepared using Sh protocol. Lane 2 & 4 are wild-type and heterozygote lysates prepared using protocol R. In both cases the level of ER is higher in the Heterozygotes than wild-type but the difference is much more pronounced in the lysates obtained using protocol R.

I confirmed that the level of ER in *Tgfβ1* C57Bl6/129SV heterozygotes was higher (**Figure 5**) than in the wildtypes by Western blot analysis of MG lysates. This is consistent the results in the immunohistochemistry studies previously done on the mammary glands of these animals.

I have just completed my first immunostaining experiment on (TGF-β1 +/+) and heterozygotes (TGF-β1 +/-) in a Balb/c background. Frozen cryosections of mammary gland on gelatin coated cover slips and were briefly brought to room temperature, fixed and blocked in supernatant from a casein/PBS solution (pH 7.4) for 60 minutes. The tissues were then incubated for a pre-determined time in primary antibodies diluted in blocking buffer after which they were sequentially incubated in secondary antibodies with fluorescine and Texas Red respectively. Nuclei are counter stained with DAPI. Images are then acquired on a Zeiss Axiovert equipped with epifluorescence.

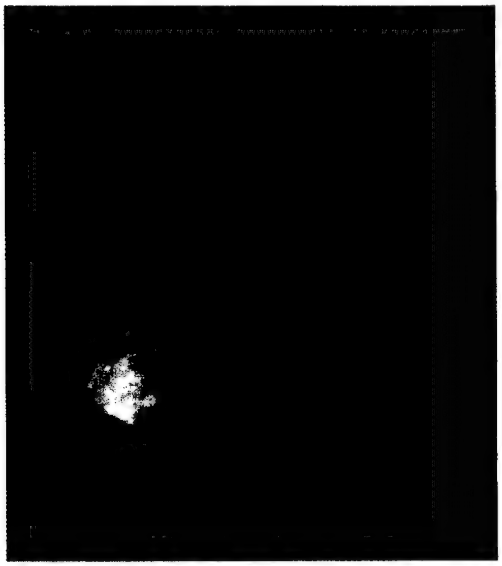


Figure 7 ER alpha (green) and Ki67 (red) are rarely co-localized (yellow) in mammary epithelial nuclei (blue).

Very few (approximately 1.5%) cells staining for ERα co-localized with Ki67 staining indicating that the ER+ cells infrequently enter the cell cycle. I will use this method in *Tgfβ1* Balb/c heterozygotes to test our hypothesis that depletion of TGF-β1 leads to increased proliferation of ER+ epithelial cells.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research and training.

Part I of this report: Mastered the following techniques

- Ishikawa assay that can be applied to asses the estrogenicity of test agents,
- MTS cell proliferation assay, a robust non-radioactive techniques for assessing cell proliferation (anchorage dependent growth) and viability cells,
- Soft Agar assay, an *in vitro* technique useful for evaluating anchorage independent growth of cancer cells to assess the potential tumorigenicity.
- The ERE-luciferase reporter assays
- The RNAse protection assay
- DPPH assay a robust bench top assay for assessing preliminary antioxidant activity.

Part II of this report: The ongoing research

- Established MCF-7 culture in serum free media.
- Have established a western blot protocol for the analysis of ER in mouse mammary glands and ready to go on to assessing the experimental samples.
- Have mastered the Immunohistochemistry techniques

REPORTABLE OUTCOMES:

Outcomes that have resulted from the research in Part I of this report include:

1) Manuscript titled "Black Cohosh: A Menopausal Herbal Remedy Does Not Have Estrogenic Activity" This was drafted and is currently being finalized by Dr Ruth Lupu for publication.

2) Abstract No 134: Titled "Black Cohosh *(Cimicifuga racemosa)* does not have any estrogenic activity" (attached as Appendix I) for a poster presented at the International Scientific Conference on COMPLEMENTARY ALTERNATIVE & INTERGRATIVE Medicine Research that took place in Boston in April 12-14, 2002 at the Boston Marriot Copley Place, Boston MA.

CONCLUSIONS

For Part I of this report: During my training period the data was generated on two Phytomedicines, namely, Black Cohosh (*Cimicifuga racemosa*) and on the herbal components of a modified Chinese herbal formula. These data are additions to the body of knowledge on phytomedicines which is still only scanty for many of the natural/phytomedicines already available in the market and being used by many American women as neutriceuticals or as alternative therapies for breast cancer treatment and to alleviate menopausal symptoms. Although many such products are readily available for over the counter dispensing and, in health food stores, they are not necessarily as safe as believed and any additional information that can be obtained through rigorous scientific research would be valuable in evaluating the efficacy and potential toxicity of these products.

For Part II of this report: To date, my preliminary data conforms to the hypothesis that TGF-β is restraining ER+ cells from proliferating. Further work will concentrate on specific roles of this cytokine at different stages of mammary gland development and during the initiation, and progression of carcinogenesis.

If ER-positive and anti-estrogen responsive breast tumors can spontaneously progress to an ER-negative and anti-estrogen-resistant phenotype, thereby becoming deadly metastatic diseases, then the mechanism by which breast cancer appears to progress from an ER-positive to an E2-negative phenotype is of considerable clinical importance. Our studies investigating the role of TGF-β hopefully will shade some light on this process.

At a personal level this DOD BCRP post-doctoral fellowship has enable me to learn and master skills and techniques as well as acquire knowledge in the area of breast cancer research. Specifically I have been introduced to techniques in the biochemistry, cell biology, molecular biology and animal physiology that are essential for cancer research and will greatly strengthen my capability to do research. Together with my training in pharmacognosy I hope to be able to better contribute in breast cancer research from this perspective.

Being at LBNL through the support of this fellowship has also provided me the opportunity to meet with other breast cancer researchers and learn about different areas of research that have greatly widened my knowledge in this subject.

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APPENDICES:

Appendix I:

Abstract: Black cohosh *(Cimicifuga racemosa)* does not have any estrogenic activity.

Appendix II:Curriculum vitae for the trainee: Hellen Oketch-Rabah, Ph.D

134 Black cohosh (*cimicifuga racemosa*) does not have any estrogenic activity

HA Oketch-Rabah, I Mehemi, MS Tsai, E Atlas, E Kennelly, P Nuntanakorn, F Kronenberg, R Lupu

Presenting Author: HA Oketch-Rabah

Purpose: Black cohosh (BC) is currently being taken by many American women to alleviate menopausal symptoms such as hot flashes. Estrogen, the primary treatment for hot flashes, is not recommended for women at high risk for breast cancer, or for breast cancer patients and survivors. The mechanism by which BC reduces the frequency of hot flashes is still unknown. The goal of our studies was to determine whether there is any estrogenic-like activity in extracts derived from BC, and to determine the safety of BC for women who should not take or choose not to take estrogen. The black cohosh extract used in these studies is currently under clinical trial for hot flashes at Columbia University. **Methods:** a) Extract preparation: Extracts from BC roots and rhizomes were made in hexane (BC1), ethyl acetate (BC2) and water (BC3), by sequential solvent-solvent partitioning of an aqueous-methanol BC crude extract, b) Ishikawa cell assay: Estrogenic activity was determined by the Ishikawa cell assay that measures the estrogenic activity of a compound (s) by inducing endogenous alkaline phosphatase (AP) enzyme activity, c) Transcriptional activation assay: At the molecular level, we tested the ability of the BC extracts to modulate the estrogen receptor (ER) function as evaluated using the ERE-luciferase reporter assay, d) RNAse Protection assays (RPA): We assess the extracts' ability to regulate the mRNA expression of E2-regulated genes, ER-α, PgR and pS2. These genes are regulated by synthetic estrogen and by genistein in ER-expressing breast cancer cells, e) Anchorage-dependent and -independent growth assays: We tested the ability of extracts derived from BC to induce the growth of breast cancer cells in anchorage-dependent and -independent assay. ER+ breast cancer cells were used for these assays. The assays were performed using concentrations of 0-20mg/ml. **Results:** In the Ishikawa cells based assay, the BC extracts did not enhance the AP activity, indicating no estrogenic activity. In addition, none of the BC extracts induced either ERE activity or regulation of known estrogen-regulated genes. By contrast, the synthetic estradiol (E2) significantly increased ERE-activity and regulated the expression of E2-regulated genes in breast cancer cells that express ER. Finally, we demonstrated that neither extract of BC had estrogenic effect on the growth of ER-expressing breast cancer cells. Control E2 significantly induced cell proliferation and colony formation. **Conclusion:** All of our results determine that no estrogenic activity is present in any of the BC extracts tested in our laboratory. Therefore, BC roots and rhizomes appear safe for use as an herbal remedy for the treatment of hot flashes in women for whom

RESUME

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RESEARCH INTERESTS:

My research Interest is in breast cancer research. Specifically understanding the biology of the disease and prospecting in the Plant Kingdom for natural compounds (secondary metabolites) with anticancer properties. **Particularly interested in the development of partially purified phytomedicines for the treatment of breast cancer** as well as pure natural compounds that could be developed into drugs for the treatment of breast cancer and female conditions such as post-menopausal symptoms.

EDUCATIONAL BACKGROUND:

1993-1996	Philosophy Doctorate, Pharmacognosy Royal Danish School of Pharmacy-DENMARK
Feb.-June, 1993	Diploma in Research Methodology- DENMARK
1988-1992	Master of Science, Plant Biochemistry and Physiology Kenyatta University-KENYA
1983-1986	Bachelor of Education, Science. Kenyatta University -KENYA
1980-1981	East African Advanced Certificate of Education Limuru Girls' School-KENYA
1976-1979	East African Certificate of Education Kapsabet Girls' High School-KENYA

RESEARCH AND TEACHING EXPERIENCE:

Duration	*Job title*	*Organization*
May 2001-todate	**Post Doctoral Research Fellow**	University of California, Lawrence Berkeley National Laboratories, Life Sciences Division. Molecular biology of breast cancer and biological activities of phytomedicines with potential as drugs for the treatment of breast cancer and post-menopausal symptoms.
April 2000 -May 2001	**Senior Lecturer**	Department of Pharmacology and Pharmacognosy Faculty of Pharmacy, University of Nairobi
1997 Nov-2000	**Lecturer**	Department of Pharmacology and Pharmacognosy Faculty of Pharmacy, University of Nairobi
1988- 1997	**Snr. Research Scientist**	Phytochemistry department National Museums of Kenya
1987-1988	**Research Assistant**	Molecular Genetics Department National Museums of Kenya.
1986-1987	**High School Teacher**	Kisumu Girls' High School

Teaching Experience:

2000-May-2001 **Senior Lecturer** of Pharmacognosy in the Department of Pharmacology and Pharmacognosy, Faculty of Pharmacy at the University of Nairobi.

Duties: teaching, conducting tutorial and lab classes, supervising student projects at undergraduate and graduate level, and examining Pharmacy undergraduate students in Pharmacognosy.

Administrative: Acting Chairman of Department in the absence of the Chairman. Attending faculty board meetings.

1997-May 2001 **Part-Time Lecturer** of Pharmacognosy at the Medical Training college (MTC) in Nairobi. Teaching Pharmacy Technician trainiees.

1997-April 00 **Lecturer of Pharmacognosy** in the Department of Pharmacology and Pharmacognosy, Faculty of Pharmacy at the University of Nairobi.

1998-1999 External project supervisor for two Master of Pharmacy students at the Royal Danish School of Pharmacy in Denmark.

Student Supervision:

Undergraduate final Year B.Pharm students:

Student Name	*Project*
Kaburi Albert Ndwiga(2000)	Antimicrobial activity of *Tylossema fassoglensis* root tuber.
Catherine N. Mburu (1999)	A Literature Review, Activity and Composition of *Mondia whitei*

Elishiba Mwangi (1998) Biological Activity of *Pittosporum viridiflorum*

RESEARCH ACTIVITIES

1997-1999

Research Project Proposal Development

Formulated several research project proposals, most of which have been funded as detailed in the sub title "**RESEARCH GRANTS**". We currently have a research group in the faculty of Pharmacy, constituted of members of the Department of Pharmacology & Pharmacognosy and the Department of Pharmaceutical Chemistry actively researching on Botanical antimalarials.

Research activities

Established a malaria parasite culture for use in the *in vitro* testing for antimalarial activity. Ethnobotanical and clinical surveys are ongoing to help select potential antimalarial plants. Several compounds with antimalarial activity have already been isolated from *Maytenus senegalensis* and *Todallia asiatica.*

1993-1996 Did my Philosophy Doctorate studies by course work and research. My research project was entitled "**Antimalarial and Antileishmanial Compounds from Kenyan Medicinal Plants.** The research involved, collecting ethnopharmacological information, screening of crude plant extracts of different polarities for activity against the parasites causing the two diseases. The most active extracts were subjected to bioactivity-guided fractionation to isolate the compound(s) responsible for activity. spectroscopic studies were then carried out to structurally identify the isolated compound(s). The *in vitro* antiplasmodial and antileishmanial activity of the isolated compounds was also determined.

1988-92 As a research Scientist at the National Museums, my duties included developing Research proposals for funding of Departmental projects. Screening plants for *in vitro* Antiprotozoal, antibacterial, molluscicidal and cytotoxic activity. Phytochemical screening of plant extracts using TLC and HPLC in order to identify the compounds responsible for bioactivity in the plant extracts.

RESEARCH GRANTS:

Foundation/ Year	*Project*	*Amt. US $*
EarthWatch (1999 todate)	Medicinal Plants of Kenya (Field work only)	50,000.00
WHO/TDR(2000, April Year 2)	Botanical antimalarial drugs Research	84,500.00
DFG German Foundation (July1999)	Bioactive compounds from the Kenyan Flora	65,000.00
WHO/TDR(1999, June)	Botanical antimalarial drugs Research	78,000.00

PUBLICATIONS:

1) Petrine W. Petersen, Lise Andersen, **Hellen A. Oketch-Rabah,** Vicki Clausen and Jerzy W. Jaroszewski. **Cyclopentenoid cyanogydrin glucosides of some Flacourtiaceae**. Gynocardin and cyclopentenylglycine in Rawsonia lucida.Biochem Syst Ecol. 2001 Feb 1;29(2):219-222.

2) **Hellen A. Oketch-Rabah**, Julius W. Mwangi , John Lisgarten, Edward K. Mberu. **A new Antiplasmodial coumarin from *Toddalia asiatica* roots.** *Fitoterapia* 2000, **71**: 636-40.

3) **H. A. Oketch-Rabah**, S. F. Dossaji, E. K,. Mberu **Antimalarial activity of some Kenyan medicinal plants**. *International Journal of Pharmaceutical Biology (Formerly International Journal of Pharmacognosy)* **1999** Vol. 37, No 2 329-334.

4) J.W Mwangi and **H. A. Oketch-Rabah. Traditional Herbal Medicine in Kenya.** *The Pharmaceutical Journal of Kenya* Vol 10 No. 1 **1999**, 22-24.

5) **H. A. Oketch-Rabah. Phytochemical constituents of the Genus *Asparagus* and their biological activities** *Hamdard Medicus*, **1998** Vol XLI, No 2, 33-43.

6) **H. A. Oketch-Rabah** and S. F. Dossaji. **Molluscicides of Plant origin: Molluscicidal activity of some Kenyan medicinal plants.** *South African Journal of Science* **1998,** *94,* 299-301.

7) **H. A. Oketch-Rabah**, S. Brøgger Christensen, K. Frydenvang, S. F. Dossaji, T.G. Theander, C. Cornett, W.M. Watkins, A. Kharazmi, and E. Lemmich. **Antiprotozoal Properties of 16,17-Dihydrobrachycalyxolid from *Vernonia brachycalyx***. *Planta Medica* **1998,** 64 (6) 559-562.

8) **H. A. Oketch-Rabah**, S. F. Dossaji, S. Brøgger Christensen, K. Frydenvang, E. Lemmich, C. Cornett, C.E. Olsen, M. Chen, A. Kharazmi, and T. Theander. **Antiprotozoal Compounds from *Asparagus africanus***. *Journal of Natural Products* **1997**, *60*, 1017-1022.

9) **H. A. Oketch-Rabah,** S. Brøgger Christensen, S.F. Dossaji, C. Ming, C.E. Olsen, C. Cornett, T.G. Theander, A. Kharazmi, and E. Lemmich. **Two new isomeric 5-methylcoumarins with antiprotozoal propterties from *Vernonia brachycalyx* Hoffm.** *Journal of Natural Products* **1997**, *60*, 458-461.

10) **H.A. Oketch-Rabah. Leaf Compounds in Potential Plantation species of Kenyan Aloes**. *Journal of Herbs Spices and Medicinal Plants* **1996**, 4(3) 25-34.

11) N.J. Georgiadis, P.W. Kat, H.A. Oketch, and John Patton. **Allozyme Divergence within the Bovidae**. *Evolution* **1990**, *44*, 2135-2149.

Theses

1) **H. A. Oketch-Rabah**. "Antimalarial and Antileishmanial Compounds from Kenyan Medicinal Plants". Ph.D Thesis. The Royal Danish School of Pharmacy, December, **1996.**

2) **H. A. Oketch Hellen** "A Phytochemical Investigations of three species of Kenyan Aloes selected for possible commercial exploitation". M.Sc. Thesis, Kenyatta University Nairobi, **1991.**

Articles in Preparation

1) **A MENOPAUSAL HERBAL REMEDY, BLACK COHOSH (BC), IS NOT ESTROGENIC.** Oketch-Rabah H. A, Mehemi I, Tsai MS, Atlas E., Kennelly E., Nuntanakorn P., Kronenberg F., and Lupu R.

2) Rasonaivo P. and **H.A. Oketch-Rabah** "Pre-clinical considerations on antimalarial phytomedicines. Part I: efficacy evaluation". ***In preparation.***

3) J.W. Mwangi, G.N. Thoithi, **H.A. Oketch-Rabah**, I.O. Kibwage. Constituents of the Essential Oils of *Cymbopogon afronardus*. ***In preparation.***

4) **H.A. Oketch-Rabah,** The status of Biological Evaluation of African Plants. ***In preparation.***

Publications in Conference Proceedings

1) **H.A Oketch-Rabah** and J.W mwangi, Medicinal Plants and Traditional Medicines: Can they contribute in the malaria control? IIIrd Pan-African Malaria Meeting, Nairobi, Kenya, 21-14 june 1998.

2) **H.A, Oketch-Rabah**; E. Oduol; M. A Oluka; and D. Nyamwaya, Use of traditional and Pharmaceutical medicines in Kenya. The case of Kisumu and Rachuonyo Districts in Luo Nyanza Province. Workshop on People and Medicines in East Africa. Mbale, Uganda 16-20, November 1998.

3) **H.A. Oketch-Rabah**, S.F. Dossaji. Molluscicidal Activity of some Kenyan Medicinal Plants. Workshop on Biochemical Pathways in Parasites of Medical Importance. Special Programme For Research and Training in Tropical Diseases, World Health Organization. 14th -16th January, 1998.Cape Town, South Africa.

4) **H.A. Oketch-Rabah,** S.Brøgger Christensen, S.F. Dossaji, C. Ming, C.E. Olsen, C. Cornett, T.G. Theander, A. Kharazmi, and E. Lemmich. Antimalarial compounds from *Vernonia brachycalyx* activity of some Kenyan medicinal plants. Proceedings of the 7th NAPRECA Symposium 18th - 23st August, 1997, Dar-es-Salaam, Tanzania.

5) **H.A. Oketch-Rabah,** S.Brøgger Christensen, S.F. Dossaji, C. Ming, C.E. Olsen, C. Cornett, T.G. Theander, A. Kharazmi, and E. Lemmich. Phytochemical prospecting-Leishmanicidal activity of selected Kenyan medicinal plants. Proceedings of the 6th NAPRECA Symposium, 15th -21st September 1995, Kampala, Uganda.

6) **H.A. Oketch-Rabah,** S.Brøgger Christensen, S.F. Dossaji, C. Ming, C.E. Olsen, C. Cornett, T.G. Theander, A. Kharazmi. and E. Lemmich. A novel steroidal sapogenin from *Asparagus africanus* roots. Proceedings of the 6th NAPRECA Symposium, 15th -21st September 1995, Kampala, Uganda.

7) **H.A.Oketch,** T.S.F. Dossaji, and L. E. Newton. Phytochemical investigation of Kenyan Aloes for commercial preparations. Proceedings of the 4th NAPRECA Symposium 15th - 21st Dec. 1991, Addis Ababa, Ethiopia, Pg 85.

Workshops and Conferences attended & papers presented

1) Black Cohosh *(Cimicifuga racemosa)* does not have any estrogenic activity A poster presented at the International Scientific Conference on COMPLEMENTARY ALTERNATIVE & INTERGRATIVE Medicine Research that took place in Boston in April 12-14, 2002 at the Boston Marriot Copley Place, Boston MA.

2) Strategies in the Search for New Antiprotozoal Drugs. ***Presented at the* Drug Discovery Research (DDR), WHO/TDR Scientific Working Group Meeting on Traditional Medicine and Pharmaceutical Medicine perspectives on Natural Products for the Treatment of Tropical Diseases** Geneva, August 28th to 30th, 2000.

3) Malaria Research and Reference Reagent Resource Center (MR4) workshop Handling and Managing Biological Materials. March 2-4,2000 in Ouagadougou, BURKINA FASO.

4) MIM/TDR Principle Investigators meeting, 6th- 8th March 2000 in Ouagadougou, BURKINA FASO. **R & D of New Botanical Antimalarial Agents in East Africa. Project 990096, First Year Progress report.**

5) Research Initiatives on Traditional Antimalarial Medicines (RITAM), 8th-11th December 1999 in Moshi, Tanzania.TARGETS FOR RESEARCH: Old medicines a potential source of new drugs *Representing Botanical Antimalarial Drug Development- the Kenya Group.*

6) African Women in Science and Engineering, 29 Nov-4 Dec. 99, Nairobi, KENYA. **Barriers to Women's Advancement in Academic Sciences and Engineering.**

7) Natural Products Research in three continents (Africa, Asia and Latin America), 16th -19th November, 1999, Montevideo,URUGUAY. **Status of Biological Evaluation of African Plants: *Successes, challenges and Prospects.***

8) Earth Watch Principle Investigators Meeting, 11th-14th November, 1999, BOSTON, USA. **Medicinal Plants of Kenya. *Progress of an EarthWatch Sponsored project.***

9) 8TH Natural Products Research in East and Central Africa, NAPREACA Symposium , 8-13thAugust, '99, Gabarone, BOTSWANA. **Preliminary *in vitro* antiplasmodial activity of some plants extracts used traditionally in Kenya for the treatment of malaria**

10) IDRC workshop, "What works in Development", April, 1999, Nairobi, KENYA. **What can the Research Scientists Contribute in the developing countries?**

11) Earth Watch Principle Investigators Meeting, 15th -19th October, 1998, BOSTON, USA. **Proposal on Medicinal Plants of Kenya: Western Kenya Rachuonyo District.**

12) IIIrd Pan-African Malaria Meeting, Nairobi, Kenya, 21-14 June 1998., Nairobi, KENYA. **Medicinal Plants and Traditional Medicines: Can they contribute in the malaria control?**

13) Workshop on People and Medicines in East Africa. Mbale, 16-20,Nov., 1998, Mbale, UGANDA. **Use of traditional and Pharmaceutical medicines in Kenya. The case of Kisumu and Rachuonyo Districts in Luo Nyanza Province**

14) WHO Conference on Biochemical Pathways in Parasites of Medical Importance. Special Programme For Research and Training in Tropical Diseases, World Health Organization., 14 -16 Jan., 1998, Cape Town, South Africa. **Molluscicidal compounds from Kenyan Plants.**

15) Regional Workshop on Medicinal Plants and Traditional Medicine in Cape Town, South Africa, 14-18 April, 1998. **Participatory Research and Involvement of local Communities and Traditional Healers: Potential benefits and pit falls.**

16) Ph.D. Lecture (viva) at The Royal Danish School of Pharmacy, 5 December 1996. **Kenyan Medicinal Plants: A source of newAntiprotozoal Compounds.**

17) Lecture at Copenhagen Drug Resistance meeting, September 1996. **Antimalarial compounds from Kenyan Medicinal Plants.**

18) A lecturer presented at Novo Nordisk Scientific Meeting, August 1996. **Antiplasmodial and Antleishmanial coumarins from *Vernonia brachycalyx* roots.**

19) Presented at Scientific meetings at the Copenhagen University Hospital in July and August 1996. **Antileishmanial and Antiplasmodial activity of compounds from *Asparagus africanus* and *Vernonia brachycalyx***

20) Lecture at NAPRECA Summer School in Madagascar, September 1995. **A search for Antileishmanial compounds from medicinal plants.**

OTHER COURSES:

1) 1993-1996 **Courses during the Ph.D. study at the** Royal Danish School of Pharmacy (DENMARK)

Department of Medicinal Chemistry
i) Interdisciplinary course on research theory and research methods
ii) Sample preparation and separation techniques in bio-analytical chemistry
iii) Teaching and learning: Theory and practice

iv) Academic writing in English

2) Mar-June 1993 Diploma in Res. Methodology at Danish Bilharziasis Laboratory (DBL) in Denmark.

i) **Eidemiology and Control of Tropical Vector-borne diseases**
Research design, implementation and evaluation.

ii) **Project Management**: Capabilities for project planning and reporting with emphasis on Logical Framework Approach (LFA); Introduction to personal computers (word processing and graphic programs); literature search (computerized literature databases and handbooks; budgeting, contract design and book keeping, project budgets); report preparation; oral presentations.

iii) **Data management and statistics:** Theory and applications of statistical methods in
handling of scientific data; use of SPSS/PC+ and other computer graphic packages.

iv) **Research program planning:** Preparation of project proposals and project implementation plans (objectives, methods, sample size, recording forms, budgets, etc.)

3) Information Technology and Computing (self-taught): MSDOS, MS Word, Access, Excel, Powerpoint, Microsoft Publisher, ChemWindows, ReferenceManager, Grafit. Microsoft Internet Explorer and Netscape and other Internet Information Services.

Membership to SOCIETIES

1. Member of American Society of Pharmacognosy (ASP).
2. Third World Organization for Women in Science (TWOWS).
3. African Women in Science and Engineering (AWSE).

REFERENCES

Available on request

centrations was used as a positive control. After 48 hours, cellular proliferation was assessed by measuring the conversion of formazan dye from a tetrazolium salt by metabolically active cells. Both cell lines responded to both lovastatin and RYR extract with significant inhibition of proliferation ($p < 0.01$). We conclude therefore Chinese Red Yeast Rice has the potential to have anticancer activity when used as a dietary supplement.
Corresponding Author: Audra V Lembertas, alembert@ucla.edu, (310) 825-2063

131 Pharmacological actions of a Chinese herbal formula used for seasonal allergic rhinitis (SAR)
GB Lenon, CCL Xue, CG Li
Presenting Author: GB Lenon
A Chinese herbal medicine formula has been proven its effectiveness in relieving symptoms of seasonal allergic rhinitis SAR during randomised clinical trial (Xue et al., 2000). However, the mechanism of the actions of this SAR formula (SARF) has not yet been elucidated. In this study, we investigated the effect of SARF on responses induced by various agents *in vitro*. In isolate tracheal preparations from rat or guinea-pigs, the responses to acetylcholine (10 μM), carbachol (1μM), substance P (0.1-10μM), 5-HT (1μM), prostaglandin E2, leukotriene C4 or histamine (0.1-30μM) were not significantly affected by SARF (0.04-1.0mg/ml). In contrast, contractions elicited by compound 48/80 (25μg/ml) in both tissues were significantly inhibited by SARF. The responses in the presence of SARF (0.4 mg/ml) were 62.6±8.6% (n=14, rat) and 36.3±9.2% (n=8, guinea-pig) compared to the control responses (91±8.6%, n=14, rat and 59.6±7.6%, n=8, guinea pig, respectively). In isolated rat aortic ring preparations, responses to endothelium dependant and independent relaxants acetylcholine and nitric oxide (NO) donor, sodium nitroprusside (SNP) respectively were not significantly affected by SARF. However, relaxations to L-arginine in lipolysaccharide-treated and endothelium denuded preparations were significantly inhibited by SARF. The maximal responses to L-arginine in the presence of SARF (0.4 mg/ml) were reduced to 17.9±4.1% (n=5) compared with the control response 92.5±5.7% (n=12). In addition, the release of leukotriene B4 (LTB4) induced by calcium ionophore in porcine neutrophils was also significantly inhibited by SARF (the release of LTB4 was 82.7±25.9 ng (n=4) in the presence of SARF (1ug/ml-1001ug/ml) compared with the control 142.8±14.2 ng (n=8). These findings indicate that SARF may have multiple pharmacological actions including the inhibitions of inducible NO synthase and the release of inflammatory mediators from target cells. Xue CCL, Thien FCK, Jamison J & Zhang JJS. (2000). Allergy and Clinical Immunology International, Suppl 2, 73.
Corresponding Author: CG Li, Chunguang.li@rmit.edu.au, 613 99257036

132 The effects of cold and heat property herbal formula on collagen-induced arthritis in rats
Shao Li, Yongyan Wang, Yinqi Hu, Aiping Lu
Presenting Author: Aiping Lu (Institute of Basic Theory, China Academy of Traditional Chinese Medicine)
It has been known that rheumatoid arthritis can be treated by two opposite approaches with heat or cold property herbal formula in Chinese medicine. This study is aimed to explore the differences and mechanisms of both approaches in collagen induced arthritis (CIA) rats. Qingluo Yin (QLY) is aimed to expel the pathogenetic heat with cold property in herbal formula, and consists of Radix sophorae flaverscentis, Cortex phellodendri, Caulis sinomenii, and Rhizoma dioscoreae hypoglaucae. Wenluo Yin (WLY) is aimed to expel the pathogenetic cold with heat property in herbal formula, and consists of Radix aconiti lateralis preparata, Rhizoma atractylodis macrocephalae, Ramulus cinnamomi, and Herba selaginellae. The 60 Wistar rats were from China Academy of Medical Sciences, and divided into three groups, control, QLY, and WLY, with 20 in each group. The CIA was induced by immunization of emulsified collagen II and complete adjuvant. The TNF-α, IL-6 and IL-1β in serum and ACTH and cortisol in plasma were tested by Radioimmunoassay. The results showed that both QLY and WLY can reduce the score of pain ($P<0.01$) and swelling of ankle ($P<0.01$), can resist the infiltration of inflammatory cells, reduce newly capillary and pannus formation ($P<0.05$ 0.01), and can decrease the level of TNF-α($P<0.01$- 0.05). However, both of the formula showed some differences on the effect. WLY had obvious influence on reducing the level of ACTH and cortisol at time of 6:00 am, 12:00 am and 24:00 pm ($P<0.05$, $P<0.01$). While QLY could increase the level of cortisol at 18:00 pm ($P<0.05$) and decrease at 6:00 am ($P<0.05$), the formula maintained the circadian rhythm of cortisol. Also QLY can induce or adjust the circadian rhythms of IL-β,IL-6 and TNF-α, while WLY can not. Our results suggest that QLY and WLY, with different property in herbal medicine, can make different effects in CIA rats by changing the level and pattern of ACTH, cortisol, IL-1β,IL-6, and TNF-α.
Corresponding Author: Aiping Lu, Catcm@public.bta.net.cn, 0086-10-64-76064

133 Phytochemicals in the California avocado: preliminary evidence for inhibition of prostate cancer cell growth
Qing-Yi Lu, Qifeng Zhang, Vay Liang Go, David Heber
Presenting Author: Qing-Yi Lu (UCLA School of Medicine)
Avocado has been considered primarily a source of monounsaturated fats in the diet. However, little is known regarding its other phytochemical contents. The yellow-green color of the avocado prompted us to initiate additional studies of the avocado composition. The purposes of the study were to determine concentrations of carotenoids, retinol, and vitamin E in the avocado and to examine the potential of avocado extract to inhibit PC-3 prostate cancer cell growth. Carotenoids and fat-soluble vitamins were determined in the California Hass avocado (Mission Produce Inc., Oxnard, CA) using high-pressure liquid chromatography. Seasonal and sample-to-sample variations were measured and considered in this analysis. Prostate cancer cells were quantitated using 3H-thymidine after 72-hrs incubation. Avocados were found to contain more lutein (293 Gug/100g) than any other fruit, and to contain significant amounts of vitamin E (3205 ug/100g), as well. Avocado extract was shown to reduce PC-3 prostate cancer cell line growth by 18% and 44 % at concentrations of 83 and 250 ug/ml extract, respectively. Our study indicates that avocado is a fruit which may contribute in the potential cancer prevention and is more than simply a source of monounsaturated fat.
Corresponding Author: Qing-Yi Lu, Qlu@mednet.ucla.edu, (310) 825-3126

134 Black cohosh (*cimicifuga racemosa*) does not have any estrogenic activity
HA Oketch-Rabah, I Mehemi, MS Tsai, E Atlas, E Kennelly, P Nuntanakorn, F Kronenberg, R Lupu
Presenting Author: HA Oketch-Rabah
Purpose: Black cohosh (BC) is currently being taken by many American women to alleviate menopausal symptoms such as hot flashes. Estrogen, the primary treatment for hot flashes, is not recommended for women at high risk for breast cancer, or for breast cancer patients and survivors. The mechanism by which BC reduces the frequency of hot flashes is still unknown. The goal of our studies was to determine whether there is any estrogenic-like activity in extracts derived from BC, and to determine the safety of BC for women who should not take or choose not to take estrogen. The black cohosh extract used in these studies is currently under clinical trial for hot flashes at Columbia University. **Methods:** a) Extract preparation: Extracts from BC roots and rhizomes were made in hexane (BC1), ethyl acetate (BC2) and water (BC3), by sequential solvent-solvent partitioning of an aqueous-methanol BC crude extract, b) Ishikawa cell assay: Estrogenic activity was determined by the Ishikawa cell assay that measures the estrogenic activity of a compound (s) by inducing endogenous alkaline phosphatase (AP) enzyme activity, c) Transcriptional activation assay: At the molecular level, we tested the ability of the BC extracts to modulate the estrogen receptor (ER) function as evaluated using the ERE-luciferase reporter assay, d) RNAse Protection assays (RPA): We assess the extracts' ability to regulate the mRNA expression of E2-regulated genes, ER-α, PgR and pS2. These genes are regulated by synthetic estrogen and by genistein in ER-expressing breast cancer cells, e) Anchorage-dependent and -independent growth assays: We tested the ability of extracts derived from BC to induce the growth of breast cancer cells in anchorage-dependent and -independent assay. ER+ breast cancer cells were used for these assays. The assays were performed using concentrations of 0-20mg/ml. **Results:** In the Ishikawa cells based assay, the BC extracts did not enhance the AP activity, indicating no estrogenic activity. In addition, none of the BC extracts induced either ERE activity or regulation of known estrogen-regulated genes. By contrast, the synthetic estradiol (E2) significantly increased ERE-activity and regulated the expression of E2-regulated genes in breast cancer cells that express ER. Finally, we demonstrated that neither extract of BC had estrogenic effect on the growth of ER-expressing breast cancer cells. Control E2 significantly induced cell proliferation and colony formation. **Conclusion:** All of our results determine that no estrogenic activity is present in any of the BC extracts tested in our laboratory. Therefore, BC roots and rhizomes appear safe for use as an herbal remedy for the treatment of hot flashes in women for whom estrogen therapy presents a risk.
Corresponding Author: Ruth Lupu, Rlupu@lbl.gov, (510) 486-6874

135 Electroacupuncture stimulation of hindlimb acupoints induces expression of c-fos protein in the brain pathways
Xiao-Xue Zhang, Sheng-Xing Ma, Xi-Yan Li
Presenting Author: Sheng-Xing Ma (UCLA School of Medicine)
Purpose: The expression of immediate early gene, c-fos, has been used to map the distribution of brain neurons activated by stimulation, and Fos-like immunoreactivity (FLI) serves as a marker of neuronal activity to trace the neuronal pathway. We have recently observed that neuronal nitric oxide synthase expression is predominantly increased in the gracile nucleus with electroacupuncture (EA) stimulation of hindlimb acupuncture points (acupoints) in rats. The gracile nucleus receives peripheral somatosensory afferent inputs projecting from the hindlimb. Gracile-thalamic pathway plays an important role in the central modulation of somatosympathetic and cardiovascular functions. In the present study, we examined the influence of EA stimulation on the expression of FLI in the brainstem, thalamus and cortex by using immunohistochemical technique. **Methods:** Low-frequency EA stimulation (3 Hz) was applied to the hindlimb acupoints, Jinggu and Shugu (BL 64-65), in rats anesthetized with ketamine. Rats in the sham-treated group received surgery and EA needles were placed into the acupoints without performing the stimulation. After 2 hours stimulation and sham-treatment, the animals were perfused with 4% paraformaldehyde. Sections of rat brain were examined by immunolabeling with a polyclonal antibody directed against c-fos. **Results:** Unilateral EA stimulation of BL 64-65 caused increases in c-fos immunostained cells (133±32% [mean±SE]) in the ipsilateral gracile nucleus, and (74±28%) in the contralateral sides compared with sham-treated rats ($P<0.05$, n=4). c-Fos immunostaining